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SEASONAL CHANGES IN THE THYROID GLAND IN THE MALE COBRA, *NAJA NAJA* L.

K. W. CHIU, J. G. PHILLIPS AND P. F. A. MADERSON

Department of Biology, The Catholic University of America, Washington, D. C. 20017; Department of Zoology, University of Hull, Yorkshire, England; and Department of Biology, Brooklyn College of the City University of New York, Brooklyn, New York 11210

The activity of the reptilian thyroid gland as judged by its histological appearance seems to be largely dependent on seasonal temperature and the reproductive state of the animal (see review, Lynn, 1969). Although these conclusions derive mainly from work on lizards, there is some information available on snakes. St. Girons and Duguy (1962) found that in two species of *Vipera,* the thyroid epithelial height is lowest in winter and increases to a maximum in early spring following the time of emergence from hibernation. It is low during the summer, but increases in autumn to a second peak. However, Binyon and Twigg (1965) found that *Natrix* presents a different picture from that of *Vipera.* In *Natrix,* the thyroid cell height is greatest in February, decreases steadily in July and October and is still low the following January.

In the female squamate, certain aspects of reproduction (*i.e.,* yolk deposition, ovulation and control of gestation in viviparous forms) are associated with thyroid activity (Eggert, 1935; Miller, 1955; St. Girons and Duguy, 1962; Wilhoft, 1964). In both sexes, the thyroid appears to be associated with periodic skin-shedding (see reviews by Sembrat and Drzewicki, 1936; Goslar, 1958; Maderson, 1965a; Chiu, Phillips and Maderson, 1967; Lynn, 1969). Both of these aspects of squamate biology should therefore be taken into consideration in any study of the seasonal variation in the thyroid gland.

In the present work, seasonal variation in the thyroid histology of the male cobra (*Naja naja* L.) has been studied, and an attempt has been made to demonstrate the extent to which the picture of annual thyroid activity might be affected by changes in the gland associated with the sloughing cycle.

Materials and Methods

Each month from October, 1965, through September, 1966, 5 to 7 male cobras (*Naja naja* L.) from South China were obtained via local Hong Kong sources. After decapitation, the total body weight, and the weight of the abdominal fat body were recorded. A piece of the belly-skin was prepared for histological examination and the stage of the sloughing cycle established (Maderson, 1965b).

The thyroid gland was freed from connective tissue, weighed, and fixed in Bouin's fluid for 48 hours. It was then dehydrated, cleared in chloroform, and embedded in paraffin. Serial sections through the center of the gland were cut at 7 μ, mounted and stained with hematoxylin and eosin.

The assessment of follicular cell-height was made as follows. The 15 to 25 follicles which fell along the longest axis of the gland were examined. The tallest and shortest cell in each follicle was measured with an ocular micrometer. The average of these two values was taken as the cell-height for the particular follicle, and the mean of the cell-height for 15–25 follicles was taken as the cell-height for the gland. The average of the cell-heights of all glands examined during one month was taken as the cell-height for the month.

Results

The thyroid weight as expressed on a fat free body weight was very low (11 mg%) in November prior to hibernation (Table I and Fig. 1). There was a significant increase in the weight of the gland during hibernation so that the February figure was 50% above the November figure ($P < 0.05$). Between February and June there was a steady decrease in weight (50%, $P < 0.05$). There was a rapid and significant increase (40%, $P < 0.01$) from June to July, followed by a steady decrease during the successive months August through October (see Fig. 1).

Changes in the cell-height of the gland do not correspond to weight changes (Table I and Fig. 1). In fact, when the gland weighed least in November and June, the follicular epithelium was high. There was no significant change in

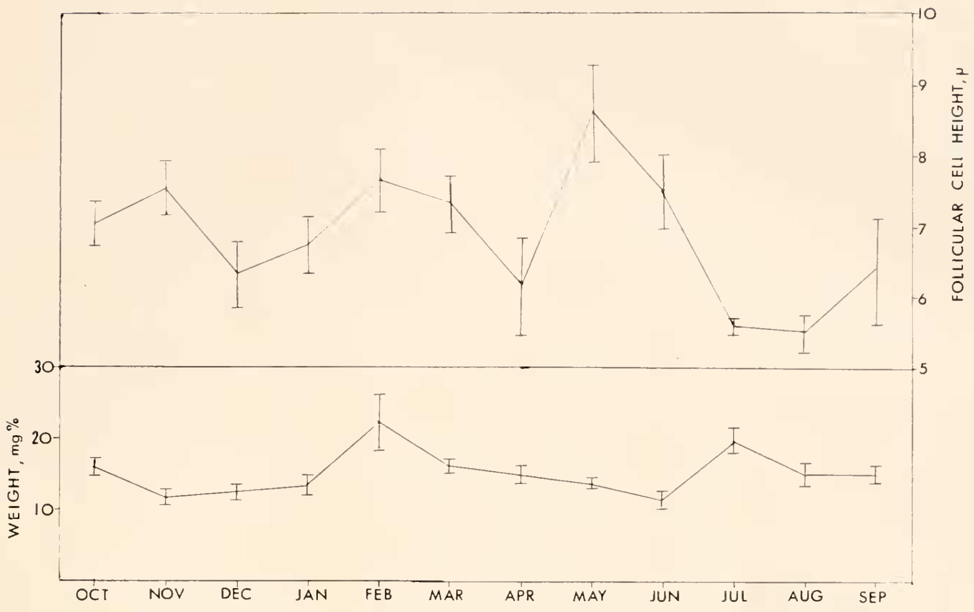
TABLE I

The weight and epithelial cell-height of the thyroid gland of the male cobra, Naja naja *L. in different months of the year*
Mean ± S.E.

Month of the year	No. of animal	Thyroid gland	
		Weight mg %+	Cell-height μ
October, 1965	5	15.58 ± 1.70	7.06 ± 0.32#
November, 1965	6	11.79 ± 1.08*	7.54 ± 0.38
December, 1965	6	12.68 ± 1.17	6.33 ± 0.46
January, 1966	6	13.59 ± 1.49	6.75 ± 0.40
February, 1966	6	22.37 ± 3.82***	7.66 ± 0.45
March, 1966	6	16.24 ± 1.00	7.32 ± 0.40
April, 1966	6	14.96 ± 1.12	6.15 ± 0.70
May, 1966	7	13.84 ± 0.51	8.62 ± 0.71###
June, 1966	6	11.67 ± 1.28****	7.50 ± 0.52##
July, 1966	6	19.92 ± 1.96**	5.58 ± 0.11
August, 1966	6	15.16 ± 1.61	5.49 ± 0.27
September, 1966	6	15.16 ± 1.33	6.40 ± 0.75

+ Fat-free body weight.
* Jul. v Nov. $P < 0.01$.
** Jul. v Jun. $P < 0.01$.
*** Nov. v Feb. $P < 0.05$.
**** Jun. v Feb. $P < 0.05$.
# Oct. v Aug. $P < 0.01$.
May v Apr. $P < 0.05$.
July v Jun. $P < 0.01$.

FIGURE 1



SEASONAL VARIATION IN THYROID EPITHELIUM, AND THYROID WEIGHT OF MALE COBRA, NAJA NAJA L. Vertical bars represent S.E.

follicular height during the hibernation period from November to April, but a 30% increase in May as compared to April ($P < 0.05$) suggests an increase in secretory activity. This increase was of short duration, being followed by a 35% decrease by July ($P < 0.01$), the cell-height showing an absolute minimum of 5.5 μ during August (compared with 8.6 μ in May). A steady increase in cell-height in subsequent months giving a 30% increase ($P < 0.05$) for October as compared to August, suggests an increase in gland activity following the inactive period during the summer.

The stage of the epidermal sloughing cycle of each animal used in the study is shown in Table II. As the cobra shows some variation in the degree of development of the alpha-layer at the time of shedding [*cf.* the lizard *Anolis carolinensis* (Maderson and Licht, 1967)], the staging is slightly different from that ascribed to *Elaphe taeniura* (Maderson, 1965b). For this reason, Table II shows no "stage 6" conditions as described by Maderson (1965b), but includes a readily recognizable "post-slough" condition when the alpha-layer is still being completed (Maderson and Licht, 1967); this is designated as Stage O. The distribution of epidermal conditions denoting the latter part of the renewal phase (Maderson, 1967) prior to shedding is of particular importance. Stages 4 and 5 (Maderson, 1965b) are represented by 0%, 33% and 33% of the animals sampled during August, July and April (the months during which thyroid epithelial height is lowest) and by 0%, 0% and 17% of the animals sampled during May, February and June (the months during which the thyroid epithelial height is greatest).

Table II

Stages of the skin and thyroid epithelial cell-heights in individual snake killed at each month

Month	Snake No.	Skin stage	Cell height μ	Month	Snake No.	Skin stage	Cell height μ
Oct.	151	5	8.15	Apr.	187	0	5.27
	152	1	6.46		188	5	6.42
	153	0	6.90		189	2	7.73
	155	5	7.28		190	3	4.74
	156	1	6.53		191	0	5.82
					192	5	6.91
Nov.	157	1	6.94		193	1	8.81
	158	1	8.53		194	2	10.85
	159	1	7.16		195	1	6.96
	160	5	6.80		196	2	7.12
	161	3	7.54		197	3	7.83
	162	1	7.74		198	3	7.27
					199	1	11.53
Dec.	163	0	5.72	Jun.	200	0	5.86
	164	0	5.66		201	1	6.95
	165	1	5.94		202	0	8.89
	166	0	8.62		203	5	8.84
	167	0	5.83		204	1	8.05
	168	0	6.24		205	0	6.41
Jan.	169	1	6.17	Jul.	206	1	5.86
	170	1	6.45		207	0	5.68
	171	0	6.80		208	2	5.46
	172	5	6.24		209	4	5.26
	173	0	8.68		210	1	5.33
	174	1	6.13		211	4	5.87
Feb.	175	1	7.68	Aug.	212	1	5.34
	176	1	7.94		213	0	5.19
	177	1	7.24		214	1	5.02
	178	1	10.77		215	2	5.31
	179	1	6.39		216	0	6.82
	180	1	5.93		217	2	5.26
Mar.	181	3	7.39	Sep.	218	0	5.75
	182	3	6.17		219	5	9.86
	183	1	8.06		220	1	6.11
	184	1	8.81		221	4	5.70
	185	1	6.95		222	4	6.56
	186	2	6.54		223	1	4.45

Discussion

It has been shown elsewhere (Maderson, Chiu and Phillips, 1969) that in snakes, thyroid activity as judged by epithelial height, increases during the latter part of the epidermal renewal phase [stages 4–5 (Table II) and (Maderson, 1965b)] and decreases sharply during the resting phase, reaching its lowest point at the beginning of the new renewal phase [stages 2–3 (Table II) and (Maderson, 1965b)]. In the present context therefore, it is important to establish whether fluctuations in the pattern of thyroid activity are related to the sloughing cycle. We suggest that the distribution of epidermal conditions representing late renewal phase (stages 4–5) as shown in Table II is not correlated with the overall pattern of thyroid activity.

The determinations of thyroid weight indicate that there is a storage phase during hibernation, a release phase in the spring, followed by a rapid storage of secretion during the summer and another period of release in autumn. The pattern of secretory activity as judged by the follicular cell-height at different times of the year is in close agreement.

The present data show that there are two peaks of thyroid activity during the year in the cobra. The first is in May just after the animal emerges from hibernation, the second is during October-November just before the animal enters hibernation. This conclusion agrees with that of St. Girons and Duguy (1962) who reported on two species of *Vipera,* but is at variance with that of Binyon and Twigg (1965) for *Natrix*. Although Binyon and Twigg's work lacks statistical analysis, their data do suggest a slight increase in epithelial cell-height (about 25%) from October to January, and this might represent the second "peak" reported for *Naja* and *Vipera.*

As *Vipera* mates twice a year, in spring when the animal emerges from hibernation, and prior to hibernation in autumn, St. Girons and Duguy (1962) concluded that the seasonal changes in gland activity are associated with mating in the male, and ovulation in the female, rather than with environmental temperature. In fact, in both sexes of *V. aspis* and *V. berus,* low thyroid activity was noted during the period of highest environmental temperature, July and August.

Lofts, Phillips and Tam (1966) showed that after emergence from hibernation in April-May, the male cobra shows spermeiogenesis and mating. As this coincides with high thyroid activity, support is found for St. Girons and Duguy's (1962) conclusions for *Vipera*. However, as cobra only mates once a year, the second peak of thyroid activity in the autumn cannot be related to sexual activity as has been concluded for *Vipera* (St. Girons and Duguy, 1962) and some lizard species (Oliver, 1955; Wilhoft, 1964). Wilhoft (1963a, b, 1964) concluded that mating activity is not related to thyroid changes in the tropical skink, *Leiolopisma*. Data relating thyroid activity to spermatogenesis in squamates is paradoxical. Spermatogenesis in the fall has been reported in *Xantusia* (Miller, 1955) and three iguanid genera (Hahn, 1964; Licht, 1966; Wilhoft and Quay, 1961). In *Xantusia* (Miller, 1955), spermatogenesis in the fall is accompanied by high thyroid activity, but this is not true for *Sceloporus* (Wilhoft, 1958; Wilhoft and Quay, 1961). In *Leiolopisma* (Wilhoft, 1963a, b, 1964) and *V. aspis* and *V. berus* (Volsøe, 1944; St. Girons, 1957; St. Girons and Duguy, 1962) where spermatogenesis is continuous throughout the year, it is not correlated with thyroid activity. However, the present data show that high thyroid activity in the cobra is correlated with testicular recrudescence in September and spermiogenesis in the following spring as reported by Lofts *et al.* (1966). In fact, these authors (Tam, Phillips and Lofts, 1967) later show two peaks of testosterone production from the male gonad which are coincident with the peaks of thyroid activity described here, but in the absence of further information on seasonal variation in androgen production in other squamates, no general conclusion regarding the relationship between the thyroid and the testis is possible.

There is a considerable body of literature relating thyroid gland activity to environmental temperature in poikilotherms (Lynn, 1969). Investigations of natural and experimental populations of lizards have shown that high temperatures are associated with high thyroid activity. However, according to the results presented here for *Naja naja,* for *Vipera* (St. Girons and Duguy, 1962) and for *Natrix* (Binyon and Twigg, 1965), snakes appear to show low thyroid activity during the summer months. To what extent this reflects fundamental differences in thyroid function between the two squamate groups, and/or behavioral mechanisms associated with thermo-regulation (see review, Brattstrom, 1965) must await further data.

Three points merit consideration as possible causes of seasonal changes in snake thyroid function. First, in lizards which remain active during winter, thyroid activity remains high, but in hibernating forms, the activity is lower at this time (see review, Lynn, 1969). Tropical forms show no significant seasonal variation (Wilhoft, 1963a, b, 1964). Second, low thyroid activity in snakes during the summer months need not necessarily be associated with the environmental temperature if the animal shows thermo-regulatory behavior (Brattstrom, 1965; Hutchison, Dowling and Vinegar, 1966). Third, the present study cannot support St. Girons and Duguy's (1962) conclusion that peaks of thyroid activity in snakes are associated with mating behavior. We conclude that increase in thyroid activity in snakes is associated with the animal's activity, both behavioral and metabolic. Thus the post-hibernation peak of thyroid activity could be associated with a variety of behavioral activities, *e.g.,* food-finding, territorial establishment and also mating. Intensive food-searching (leading to a storage of fat utilized during hibernation) in the autumnal pre-hibernation period, would possibly represent a total increase in general activity. That autumnal food-searching leads to fat storage before hibernation is suggested by the fact that hibernating animals show 10% body weight represented as fat during March, against only 0.1% during July (Chiu, unpublished data).

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Summary

1. Variations in the weight and follicular cell-height of the thyroid gland of the male cobra (*Naja naja* L.) have been studied for 12 consecutive months during a one year period.

2. Changes in the weight of the gland were not correlated with follicular cell height.

3. The epithelial height was greatest during May and October-November, lowest in August.

4. Variations in the thyroid gland activity that are associated with epidermal sloughing are not great enough to alter the overall pattern of seasonal variation.

5. It is suggested that the annual cyclic changes in the thyroid gland activity are probably related to the general activity of the male animal throughout the year and are not associated with fluctuations in environmental temperature nor, as in the female, with any specific aspect of the reproductive cycle.

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